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Multi-response optimization of factors affecting ultrasonic assisted extraction from Iranian basil using central composite design

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ABSTRACT

The present study reports on the extraction of antioxidant compounds from Iranian *Ocimum basilicum*. Central composite design (CCD) was used to investigate the effect of extraction variables on the ultrasound-assisted extraction (UAE). Three independent variables including temperature, methanol to water ratio percent, and sonication time were studied for simultaneous optimization of antioxidant capacity, total phenolic content and extraction yield. Both quantitative modeling and response surface methodology suggested that methanol to water ratio percent and extraction temperature were the most effective parameters of UAE process. However, sonication time was found out to be an insignificant factor in ultrasound-assisted extraction of antioxidant and total phenolic compounds of *O. basilicum*. The optimum conditions were determined as temperature of 59 °C, methanol to water ratio of 65.2% (v/v), and extraction time of 20 min.

1. Introduction

Antioxidants play an important role in antioxidant defense mechanisms in biological systems, protecting lipids both in cells and in food products and having inhibitory effects on mutagenesis and carcinogenesis (Sindhi et al., 2013). Synthetic antioxidants have been banned from organic production because of their suspected action as cancer promoters (Pattono et al., 2009), so attention is now focused on medicinal plants as a rich source of natural antioxidants. Many species are used for their antioxidant activity in various parts of the world.

Ocimum basilicum L., commonly known as Sweet Basil, is a culinary plant belonging to the Lamiaceae family that is a perennial herb, native to Asia (Safari Dolatabad, Moghaddam, & Chalajour, 2014). According to pharmacological studies, various *O. basilicum* extracts have antibacterial, antifungal and antioxidant activities (Vlase et al., 2014). Its aromatic leaves and flowering tops are also widely used in food industry (flavorings foods and beverages), perfumery (e.g. hair dressings, perfumes, soaps, dental creams, mouth washes) and in traditional medicine (Maggini, Kiferle, Guidi, Pardossi, & Raffaelli, 2012). Phenolic compounds constitute a major class of basil secondary metabolites that are contributed to antioxidant and anti-inflammatory activity of its extracts (Kwee & Niemeyer, 2011). Since they are not uniformly distributed in the plant and their stability varies significantly, the extraction procedure seems to be the primary determinant for the separation and recovery of antioxidants. Extraction of antioxidants from solid plant materials is most commonly done using Soxhlet, (heated reflux extraction) and maceration methods that are conventional procedures frequently used to recover phenolics from solid samples (Arceusz, Wesolowski, & Konieczynski, 2013). However, they present various shortcomings such as toxic wastes, chemical transformation of extracts, and long extraction times.

Due to these problems associated with conventional extraction procedures, an increasing demand from industries for a proper extraction method is observed. Short extraction time, efficiency, simplicity and having low cost are important parameters that are commonly considered for a suitable extraction technique (Wang et al., 2013).

In response to this growing need, a number of methods have been recently developed such as microwave-assisted extraction (MAE) (Mushtaq, Choi, Verpoorte, & Wilson, 2014), ultrasound-assisted extraction (UAE) (Picó, 2013) and techniques based on use of compressed fluids as extracting agents, such as pressurized fluid extraction (PFE) or accelerated solvent extraction (ASE) (Santos-Buelga, Gonzalez-Manzano, Dueñas, & Gonzalez-Paramas,

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2012) have been also applied in the extraction of antioxidant compounds from plant materials. Among these methods, UAE is a well-established combinatory technique that has been widely investigated lately (Braga, Seabra, Dias, & de Sousa, 2013). In comparison with conventional methods, UAE technology does not require complex instruments and is relatively low-cost. Therefore, it can be used both on a small and large scale in the phytopharmaceutical extraction and food industry. However, there is no standardized procedure that can be suitable for extraction of all antioxidants in plant materials. Therefore, the extraction procedure has to be optimized depending on the extraction method type and desired analytes.

It is necessary to consider that the highest yield is not always the sole objective of an extraction process and also the lowest consumption of nonrenewable resources and energy should be taken into account. Therefore, the optimization of those parameters is necessary to transfer experimental laboratory conditions to industrial scales. In traditional form, optimization is carried out by monitoring the influence of one factor at a time on an experimental response. This kind of optimization is called one-variable-at-a-time (OVAT) because except for one variable the others are kept constant. The main drawback of this technique is that the interactive effects among the variables studied are ignored which results in defective depiction of the parameter effects on the response. Another disadvantage of OVAT is the high number of experiments required in optimization process (Marini, 2013).

Response surface methodology (RSM) is an optimization technique which has been widely used to overcome these problems (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008). In this methodology, the relationship between the response of an analytical system and the levels of the effective factors can be represented by a surface in three dimensions that is called the response surface. Among the many classes of RSM designs, central composite design (CCD) is among the most popular methods due to its simple structure and efficiency (Correia, Gonçalves, da Cunha Jr., & Ferraresi, 2005). Based on the best of our knowledge, there are only few reports on optimization of antioxidants extraction from basil using RSM technique and pressurized liquid extraction (PLE) and maceration extraction methods (Hossain, Brunton, Martin-Diana, & Barry-Ryan, 2010; Juntachote, Berghofer, Bauer, & Siebenhandl, 2006; Vidović et al., 2012). However, no research article regarding the RSM optimization and investigation of antioxidants extraction from basil using UAE method was found. RSM has been also successfully applied to optimize and investigate the effect of extraction parameters on microwave-assisted extraction of total phenolics from *Myrtus communis* leaves (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015) and conventional solvent extraction of anti-tumor alkaloids from the stem of medicinal plant of *Berberis amurensis Rupr.* (Wu et al., 2015). Given the emerging nature of UAE technique in pharmaceutical and food industry, further researches are needed for the application of this technique as a standardized industrial method (Vilkhu, Mawson, Simons, & Bates, 2008). Once the UAE extraction procedure is optimized for antioxidant extraction, the optimal conditions could be also applied for the other *Lamiaceae* plants. Accurate assessing and monitoring of the effective parameters in UAE (extraction time, solvent, and temperature) and elucidation of the response changes with the alterations of extraction parameters, could provide a brighter insight into the UAE mechanism of action and could be applied on how to use this technology more efficiently for antioxidant extraction. In this work, RSM and central composite design were applied to investigate the optimal conditions for multi-response extraction from *O. basilicum* using UAE extraction method.

2. Materials and methods

2.1. Chemicals and plant materials

All solvents were of analytical grade and were purchased from Merck (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, sodium carbonate, and gallic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA).

The raw plant material (*O. basilicum*) was obtained from an olericulture store in Ahvaz city. *O. basilicum* leaves were thoroughly washed and air dried, away from direct sunlight. The material was then ground (using a coffee mill) for 3 min, so as to obtain the corresponding powder. This powder was consecutively passed through 0.3 mm mesh size sieve and the collected powder was stored at −20 °C in an airtight container until used.

2.2. Ultrasound-assisted extraction (UAE)

Ultrasonic-assisted extraction was performed in an ultrasonic bath (Elmasonic S60H) operated at a frequency of 37 kHz with a useable volume of 5.67 L. Three experiment parameters including temperature (20–60 °C), methanol to water ratio (in terms of methanol volume percent, 0–100%) and time of sonication (20–70 min) were considered as the affecting parameters of extraction. Extraction procedure was as follows; 0.2 g of *O. basilicum* leaf powder was extracted with 20 mL of solvent (binary mixture of methanol and water with specified composition), for the intended time and temperature ranges (Kwee & Niemeyer, 2011). After extraction, the mixture was centrifuged for 10 min at 13,000 rpm in a centrifuge followed by filtration through a Whatman No. 1 filter paper. The clear solution was concentrated to dryness with a rotary evaporator and the extract was then collected and stored in −80 °C freezer until it was analyzed.

2.3. Formulation of extraction yield (% yield)

The yield (%, w/w) from all the dried extracts was calculated as follow:

$$\text{Yield } (\%) = (W_1/W_2) \times 100 \quad (1)$$

where W_1 is the weight of the extract after evaporation of solvent, and W_2 is the weight of the plant powder.

2.4. DPPH antioxidant assay

Antioxidant capacity for the extracts, obtained from each extraction run, was determined according to the method of Sarker, Latif, and Gray (2005) with some modifications. DPPH (4.0 mg) was dissolved in MeOH (50.0 mL) to obtain a concentration of 80.0 μg/mL. Dilutions of crude extracts were made to obtain concentrations of 1000–1.6 μg/mL. Diluted solutions (1.5 mL each) were mixed with DPPH (1.5 mL) and allowed to stand for 30 min for any reaction to take place. The absorbance of these solutions was recorded at wavelength of 517 nm. At the end, the DPPH radical scavenging effect was calculated as "inhibition percentage" using the following equation:

$$\% \text{ Inhibition} = [(A_B - A_S)/A_B] \times 100 \quad (2)$$

where A_B and A_S are respectively the absorbance of the control (mixture of MeOH and DPPH solution, 1.5 mL each) and the test solutions at 517 nm.